

MODEL: (3M531) Intraperitoneally-Implanted M5076 Ascitic Sarcoma

Origin of Tumor Line: Arose spontaneously in a C57BL mouse at the Papanicolaou Research Institute, Miami, Florida in the laboratory of Dr. W. F. Dunning.

Summary of Test Procedures: 1 x 10⁶ cells of ascitic fluid are implanted i.p. in mice. I.p. test agent treatment begins one day after tumor implant and continues every fourth day for a total of four injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: C57BL/6 female mice.

Testing: B₆C₃F₁ mice (male or female).

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test, titration, and control animals in one experiment.

Source: One source for all animals in one experiment. Exceptions must be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten mice per test group.

Control Groups: A minimum of 40 control animals must be used, otherwise the number of control animals varies according to the number of test groups.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation and Testing:

Tissue:

Suspension: Use donor tumor when the abdomen becomes distended (usually Days 14-19). Using a sterile 5 ml syringe, withdraw ascitic fluid aseptically through the abdominal wall from which the skin has been removed. Use a minimum of three (3) mice. Collect at least three (3) ml of ascitic fluid. Pool fluid in a sterile glass container held in an ice bath.

Cell Count: Use physiological saline for dilutions. Use serological pipettes with rubber bulb attachment. Cell suspensions should be swirled and mixed by aspirating the solution into and out of the pipette several times before withdrawing an aliquot. Make dilutions as follows:

Suspension A: 0.5 ml of pooled ascitic fluid plus 4.5 ml of physiological saline.

Suspension B: 0.5 ml of Suspension A plus 4.5 ml of physiological saline.

Suspension C: 1.0 ml of Suspension B plus 4.0 ml of physiological saline. Suspension C is a 1:500 dilution and should be used to make the cell count as follows:

1. Agitate Suspension C and withdraw 1 ml with a white blood cell pipette.
2. Agitate and allow 3 drops to flow out, then fill both chambers of the hemacytometer.
3. Count only intact nucleated cells using either 100x or 400x magnification.
4. Assuming the use of an A0 STD or comparable hemacytometer, count 4 large squares in both chambers, being sure to establish and follow a convention for inclusion of cells that fall on lines.
5. The cell count and the dilution ratio may be read directly from the accompanying table. To use the table, divide the total number of cells in both chambers (refer to Step 4) by 2.

These values may also be calculated directly from the total number of cells in both hemacytometer chambers as follows:

$$\frac{\text{Total no. of cells in both hemacytometer chambers}}{2} \times 2.5 \times 500 \times 1000^* = \text{cells/ml of the undiluted ascitic fluid}$$

Note: Once the cell count has been determined, there is no further need for Suspensions A, B, or C. The cell suspension for inoculation will be made from the original undiluted ascites from which Suspension A was made.

The dilution factors required to prepare a suspension of cells that contain the highest cell number required in 0.1 ml may be calculated as shown:

Example:

$$\frac{10,000,000 \text{ (cells/ml required for 10(6) in 0.1 ml implant)}}{85,000,000 \text{ (cell count/ml of ascitic fluid)}} = \frac{X \text{ ml}}{10 \text{ ml}}$$

$$85X = 100$$

$$X = 1.18 \text{ ml}$$

* 2.5: Correction factor to convert count to cells per cubic ml.

500: Dilution factor.

1000: Converts cubic mm to ml.

Add 1.18 ml of ascitic fluid to 8.82 ml of physiological saline for a suspension of 1×10^6 cells in 0.1 ml.

Preparation of Cell Suspensions for Inoculation: Sterile procedures should be followed to dilute the ascitic fluid to obtain Suspension 1 and subsequent dilutions.

Suspension 1. Highest level required = 1×10^6 cells in 0.1 ml.

Suspension 2. Add 1 part of Suspension 1 to 9 parts of physiological saline = 1×10^5 cells in 0.1 ml.

Suspension 3. Add 1 part of Suspension 2 to 9 parts of physiological saline = 1×10^4 cells in 0.1 ml.

Continue for the required number of levels, adding 1 part of the last suspension to 9 parts of physiological saline.

Time: Days 14-19.

Site: Inject 0.1 ml per mouse i.p. using a 0.5 inch, 23 gauge needle with a 1 ml Tuberculine syringe. Site of injection should be chosen to avoid vital organs.

Use sufficient numbers of sterile syringes and needles so that no syringe will be refilled from the pool of donor fluid. No more than 60 minutes should elapse from the time fluid is taken from the donor until it is implanted in the recipient animals. For titrations, inoculate the lowest level first, then proceed to inoculate each higher level. The range of inoculum levels is to be from 1×10^6 to 1×10^3 cells, inclusive.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed. Administer test agent based on initial average group weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Administer test agent.

Day 9: Administer test agent.

Day 13: Administer test agent.

Day 14: Toxicity day for test animals and second animal weigh day. Control early-death day.

Day 40: Control no-take day.

Day 60: End and evaluate experiment.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 26271* at doses of 160,

*Positive control compound NSC 26271 is Cytosan. CAS RN is 50-18-0.

80 and 40 mg/kg/injection) in every experiment, the regimen for which is Q4D x 4 beginning on Day 1. The lower T/C limit for the positive control is 150%. The acceptable untreated control median survival time is 20 - 28 days.

EVALUATION: (refer to Protocols 4 and 11)

The parameter measured is median survival time. Compute average animal body weights for Day 1 and Day 14, compute T/C for all test groups with > 65% survivors on Day 14. A T/C value < 86% indicates toxicity. A negative body weight change difference (Test Minus Control) \geq 4 gms may also be used to evaluate toxicity.

CRITERIA FOR ACTIVITY:

A T/C value \geq 125% is considered necessary to demonstrate moderate activity. A reproducible T/C value \geq 150% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a non-standard dose is administered (whether due to a solubility problem or special request), and for poor suspensions.

Table of Cell Counts Determined from a 1:500 Dilution and
the Dilution Ratio for 1×10^6 Cells in 0.1 cc

From Hemacytometer ^a No. of Cells Counted	Cell Count ^b ($\times 10^7$)	Dilution Ratio ^c		
		Ascites ^d (ml)	+	Saline ^e (ml)
50	6.250	1.60	+	8.40
51	6.375	1.57	+	8.43
52	6.500	1.54	+	8.46
53	6.625	1.51	+	8.49
54	6.750	1.48	+	8.52
55	6.875	1.46	+	8.55
56	7.000	1.43	+	8.57
57	7.125	1.40	+	8.60
58	7.250	1.38	+	8.62
59	7.375	1.36	+	8.64
60	7.500	1.33	+	8.67
61	7.625	1.31	+	8.69
62	7.750	1.29	+	8.71
63	7.875	1.27	+	8.73
63	8.000	1.25	+	8.75
65	8.125	1.23	+	8.77
66	8.250	1.21	+	8.79
67	8.375	1.19	+	8.81
68	8.500	1.18	+	8.82
69	8.625	1.16	+	8.84
70	8.750	1.14	+	8.86
71	8.875	1.13	+	8.87
72	9.000	1.11	+	8.89
73	9.125	1.10	+	8.90
74	9.250	1.08	+	8.92
75	9.375	1.07	+	8.93
76	9.500	1.05	+	8.95
77	9.625	1.04	+	8.96
78	9.750	1.03	+	8.97
79	9.875	1.01	+	8.99
80	10.000	1.00	+	9.00
81	10.125	0.99	+	9.01
82	10.250	0.98	+	9.02
83	10.375	0.96	+	9.04
84	10.500	0.95	+	9.05
85	10.625	0.94	+	9.06
86	10.750	0.93	+	9.07
87	10.875	0.92	+	9.08
88	11.000	0.91	+	9.09
89	11.125	0.90	+	9.10
90	11.250	0.89	+	9.11
91	11.375	0.88	+	9.12
92	11.500	0.87	+	9.13
93	11.625	0.86	+	9.14
94	11.750	0.85	+	9.15
95	11.875	0.84	+	9.16
96	12.000	0.83	+	9.17
97	12.125	0.82	+	9.18
98	12.250	0.82	+	9.18
99	12.375	0.81	+	9.19
100	12.500	0.80	+	9.20
101	12.625	0.79	+	9.21
102	12.750	0.78	+	9.22
103	12.875	0.78	+	9.22
104	13.000	0.77	+	9.23
105	13.125	0.76	+	9.24
106	13.250	0.75	+	9.25
107	13.375	0.75	+	9.25
108	13.500	0.74	+	9.26
109	13.625	0.73	+	9.27
110	13.750	0.73	+	9.27

From Hemacytometer^a
No. of Cells
Counted

Cell Count^b
($\times 10^7$)

Dilution Ratio^c
Ascites^d (ml) + Saline^e (ml)

111	13.875	0.72	+	9.28
112	14.000	0.71	+	9.29
113	14.125	0.71	+	9.29
114	14.250	0.70	+	9.30
115	14.375	0.70	+	9.30
116	14.500	0.69	+	9.31
117	14.625	0.68	+	9.32
118	14.750	0.68	+	9.32
119	14.875	0.67	+	9.33
120	15.000	0.67	+	9.33
121	15.125	0.66	+	9.34
122	15.250	0.66	+	9.34
123	15.375	0.65	+	9.35
124	15.500	0.65	+	9.35
125	15.625	0.64	+	9.36
126	15.750	0.63	+	9.37
127	15.875	0.63	+	9.37
128	16.000	0.63	+	9.37
129	16.125	0.62	+	9.38
130	16.250	0.62	+	9.38

131	16.375	0.61	+	9.39
132	16.500	0.61	+	9.39
133	16.625	0.60	+	9.40
134	16.750	0.60	+	9.40
135	16.875	0.59	+	9.41
136	17.000	0.59	+	9.41
137	17.125	0.58	+	9.42
138	17.250	0.58	+	9.42
139	17.375	0.58	+	9.42
140	17.500	0.57	+	9.43
141	17.625	0.57	+	9.43
142	17.750	0.56	+	9.44
143	17.875	0.56	+	9.44
144	18.000	0.56	+	9.44
145	18.125	0.55	+	9.45
146	18.250	0.55	+	9.45
147	18.375	0.54	+	9.46
148	18.500	0.54	+	9.46
149	18.625	0.54	+	9.46
150	18.750	0.53	+	9.47

151	18.875	0.53	+	9.47
152	19.000	0.53	+	9.47
153	19.125	0.52	+	9.48
154	19.250	0.52	+	9.48
155	19.375	0.52	+	9.48
156	19.500	0.51	+	9.49
157	19.625	0.51	+	9.49
158	19.750	0.51	+	9.49
159	19.875	0.50	+	9.50
160	20.000	0.50	+	9.50
161	20.125	0.50	+	9.50
162	20.250	0.49	+	9.51
163	20.375	0.49	+	9.51
164	20.500	0.49	+	9.51
165	20.625	0.48	+	9.52
166	20.750	0.48	+	9.52
167	20.875	0.48	+	9.52
168	21.000	0.48	+	9.52
169	21.125	0.47	+	9.53
170	21.250	0.47	+	9.53

171	21.375	0.47	+	9.53
172	21.500	0.47	+	9.53

From Hemacytometer^aNo. of Cells
CountedCell Count^b
($\times 10^7$)Dilution Ratio^cAscites^d (ml) + Saline^e (ml)

173	21.625	.46	+	9.54
174	21.750	.46	+	9.54
175	21.875	.46	+	9.54
176	22.000	.45	+	9.55
177	22.125	.45	+	9.55
178	22.250	.45	+	9.55
179	22.375	.45	+	9.55
180	22.500	.44	+	9.56
181	22.625	.44	+	9.56
182	22.750	.44	+	9.56
183	22.875	.44	+	9.56
184	23.000	.43	+	9.57
185	23.125	.43	+	9.57
186	23.250	.43	+	9.57
187	23.375	.43	+	9.57
188	23.500	.43	+	9.57
189	23.625	.42	+	9.58
190	23.750	.42	+	9.58
191	23.875	.42	+	9.58
192	24.000	.42	+	9.58
193	24.125	.41	+	9.59

194	24.250	.41	+	9.59
195	24.375	.41	+	9.59
196	24.500	.41	+	9.59
197	24.625	.41	+	9.59
198	24.750	.40	+	9.60
199	24.875	.40	+	9.60
200	25.000	.40	+	9.60
201	25.125	.40	+	9.60
202	25.250	.40	+	9.60
203	25.375	.39	+	9.61
204	25.500	.39	+	9.61
205	25.625	.39	+	9.61
206	25.750	.39	+	9.61
207	25.875	.39	+	9.61
208	26.000	.38	+	9.62
209	26.125	.38	+	9.62
210	26.250	.38	+	9.62
211	26.375	.38	+	9.62
212	26.500	.38	+	9.62
213	26.625	.38	+	9.62

214	26.750	.37	+	9.63
215	26.875	.37	+	9.63
216	26.000	.37	+	9.63
217	27.125	.37	+	9.63
218	27.250	.37	+	9.63
219	27.375	.37	+	9.63
220	27.500	.36	+	9.64
221	27.625	.36	+	9.64
222	27.750	.36	+	9.64
223	27.875	.36	+	9.64
224	28.000	.36	+	9.64
225	28.125	.36	+	9.64
226	28.250	.35	+	9.65
227	28.375	.35	+	9.65
228	28.500	.35	+	9.65
229	28.750	.35	+	9.65
230	28.750	.35	+	9.65
231	28.875	.35	+	9.65
232	29.000	.34	+	9.66
233	29.125	.34	+	9.66

From Hemacytometer^a

No. of Cells Counted	Cell Count ^b ($\times 10^7$)	Dilution Ratio ^c		
		Ascites ^d (ml)	+	Saline ^e (ml)
234	29.250	.34	+	9.66
235	29.375	.34	+	9.66
236	29.500	.34	+	9.66
237	29.625	.34	+	9.66
238	29.750	.34	+	9.66
239	29.875	.33	+	9.67
240	30.000	.33	+	9.67
241	30.125	.33	+	9.67
242	30.250	.33	+	9.67
243	30.375	.33	+	9.67
244	30.500	.33	+	9.67
245	30.675	.33	+	9.67
246	30.750	.33	+	9.67
247	30.875	.32	+	9.68
248	31.000	.32	+	9.68
249	31.125	.32	+	9.68
250	31.250	.32	+	9.68
251	31.375	.32	+	9.68
252	31.500	.32	+	9.68
253	31.625	.32	+	9.68
254	31.750	.31	+	9.69
255	31.875	.31	+	9.69
256	32.000	.31	+	9.69
257	32.125	.31	+	9.69
258	32.250	.31	+	9.69
259	32.375	.31	+	9.69
260	32.500	.31	+	9.69
261	32.625	.31	+	9.69
262	32.750	.31	+	9.69
263	32.875	.30	+	9.70
264	33.000	.30	+	9.70
265	33.125	.30	+	9.70
266	33.250	.30	+	9.70
267	33.375	.30	+	9.70
268	33.500	.30	+	9.70
269	33.625	.30	+	9.70
270	33.750	.30	+	9.70
271	33.875	.30	+	9.70
272	34.000	.29	+	9.71
273	34.125	.29	+	9.71
274	34.250	.29	+	9.71
275	34.375	.29	+	9.71
276	34.500	.29	+	9.71
277	34.625	.29	+	9.71
278	34.750	.29	+	9.71
279	34.875	.29	+	9.71
280	35.000	.29	+	9.71
281	35.125	.28	+	9.72
282	35.250	.28	+	9.72
283	35.375	.28	+	9.72
284	35.500	.28	+	9.72
285	35.625	.28	+	9.72
286	35.750	.28	+	9.72
287	35.875	.28	+	9.72
288	36.000	.28	+	9.72
289	36.125	.28	+	9.72
290	36.250	.28	+	9.72
291	36.375	.27	+	9.73
292	36.500	.27	+	9.73
293	36.625	.27	+	9.73
294	36.750	.27	+	9.73
295	36.875	.27	+	9.73

Table of Cell Counts (Continued)

FOOTNOTES:

^aA. O. Spencer [®], Bright-line, Improved Neubauer, 1/10 mm deep.

^bCell count of ascetic fluid = number of cells counted per plate (or 2 plates ÷ 2) x 2.5 x 500 x 1000.

^cDilution ratio to give 10 ml of 1×10^6 cells per 0.1 ml. The dilution ratio may be used to prepare larger volumes than 10 ml by multiplying both sides by the same number; for example, 1 ml ascites + 9 ml of saline = 10 total; to increase this to 30 ml, multiply by 3; 3 ml of ascites + 27 ml of saline = 30 ml total. Conversely, the total may be reduced from 10 ml by dividing both sides by the same number.

^dAscites - undiluted taken directly from the animal.

^ePhysiological saline (do not use other diluents).